COMPARISON OF XEROSTOMIA AND SALIVARY LEVELS OF GLUCOSE, UREA AND ORAL CANDIDAL CARRIAGE IN THE SALIVA OF TYPE II DIABETICS AND NON DIABETICS: A CROSS SECTIONAL STUDY

A Dissertation submitted in

partial fulfillment of the requirements

for the degree of

MASTER OF DENTAL SURGERY

BRANCH-IX

ORAL MEDICINE AND RADIOLOGY

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

CHENNAI-600 032

2014-2017

DECLARATION BY THE CANDIDATE

TITLE OF DISSERTATION	COMPARISON OF XEROSTOMIA AND SALIVARY LEVELS OF GLUCOSE,UREA AND ORAL CANDIDAL CARRIAGE IN THE SALIVA OF TYPE II DIABETICS AND NON DIABETICS:A CROSS SECTIONAL STUDY
PLACE OF STUDY	K.S.R. Institute of Dental science and Research, Tiruchengode.
DURATION OF COURSE	3 Years
NAME OF THE GUIDE	Dr. Senthil Kumar, M.D.S
HEAD OF THE DEPARTMENT	Dr. (Capt) S. Elangovan , M.D.S

I hereby declare that no part of the dissertation will be utilized for gaining financial assistance for research or other promotions without obtaining prior permission of the Principal, K.S.R Institute of Dental Science and Research, Tiruchengode. In addition, I declare that no part of this work will be published either in print or electronic without the guide who has been actively involved in dissertation. The author has the right to reserve for publish of work solely with prior permission of the Principal, K.S.R Institute of Dental Science and Research, Tiruchengode.

Head of the Department **Guide** **Signature of Candidate**

CERTIFICATE BY THE GUIDE

This is to certify that dissertation titled **"COMPARISON OF XEROSTOMIA AND SALIVARY LEVELS OF GLUCOSE, UREA AND ORAL CANDIDAL CARRIAGE IN THE SALIVA OF TYPE II DIABETICS AND NON DIABETICS: A CROSS SECTIONAL STUDY"** is a bonafide research work done by **Dr. PRIYANKA .S** in partial fulfillment of the requirements for the degree of MASTER OF DENTAL SURGERY in the speciality of **ORAL MEDICINE AND RADIOLOGY.**

Date:

Place:

Signature of the Guide

Dr. B. SENTHIL KUMAR. M.D.S

READER

K.S.R. INSTITUTE OF DENTAL SCIENCE AND RESEARCH

TIRUCHENGODE.

ENDORSEMENT BY THE H.O.D, PRINCIPAL/ HEAD OF THE INSTITUTION

This is to certify that **Dr. PRIYANKA S**., Post Graduate student (2014-2017) in the Department of Oral Medicine and Radiology, K.S.R. Institute of Dental science and Research, has done this dissertation titled **"COMPARISON OF XEROSTOMIA AND SALIVARY LEVELS OF GLUCOSE,UREA AND ORAL CANDIDAL CARRIAGE IN THE SALIVA OF TYPE II DIABETICS AND NON DIABETICS: A CROSS SECTIONAL STUDY"** under our guidance and supervision in partial fulfillment of the regulations laid down by the **Tamilnadu Dr.M.G.R. Medical university**, Chennai-600032 for **M.D.S**., (Branch –IX) **Oral Medicine and Radiology** degree examination.

Seal and signature of H.O.D

Dr. (Capt) S. ELANGOVAN., M.D.S

PROFESSOR AND HOD

Seal and signature of Principal

Dr. G.S.KUMAR., M.D.S

PRINCIPAL

K.S.R INSTITUTE OF DENTAL SCIENCE AND RESEARCH

TIRUCHENGODE

ACKNOWLEDGEMENT

Praises and thanks to the **God, the Almighty**, for His showers of blessings throughout my research work to complete the research successfully

I am extremely grateful to my dear **Parents** for their love, prayers, caring and sacrifices for educating and preparing me for my future.

I take this opportunity to express my humble gratitude to **Dr.G.S.Kumar M.D.S, Principal,** K.S.R. Institute of Dental Science and Research for his kind permission and encouragement.

It is my pleasure to express my sincere and deep gratitude to **Dr. (Capt) S. ELANGOVAN M.D.S.,** Professor and Head of the department for his guidance and constant support and immense patience during the preparation of this dissertation and during the course of study. He has taught me to be good professional.

I would like to express my heartfelt gratitude to my guide **Dr. B.Senthil kumar M.D.S.**, Reader, Department of Oral Medicine and Radiology, for his constant support, inspiration , for giving me ideas, as how to proceed this study and for being the guiding force throughout the course of this study. Without him, the timely completion of my study would have remained an unattainable goal.

I would like to express my deepest thanks to **Dr. Suman Jaishankar M.D.S.,** Professor, for her guidance, encouragement and valuable insights. Her immense knowledge and eye for perfection has had a remarkable influence.

I would like to express my sincere gratitude to **Dr. Nazargi Mahabob M.D.S.,** Reader, for the continuous support in my study and research, constructive criticism,

motivation and enthusiasm. I thank him for helping me every time when I was in array of doubts

I thank **Dr. Deepika Rajendran M.D.S.,** senior lecturer for her constant kindness, help and encouragement in conducting this study.

My sincere thanks to **Dr. Prakash M.D.S**., Professor, Department of Public Health Dentistry, K.S.R Institute of Dental science and Research, Tiruchengode for helping me in statistical analysis of the data and its final corrections.

I thank **Dr.V.Chandrasekar Ph.D.,** Director-Research, Bio 5 innovation, Coimbatore for helping me in the laboratory procedures.

My Heartfelt thanks to my dear colleague, **Dr. Maya Chandrika.P** and my **juniors** for their unyielding support during the period of study.

A special thanks to all the patients who participated in the study. This dissertation would not have been possible without their support and co-operation.

Dedicated to my father ***Mr. A. Singaravelu****, My mother* ***Mrs. L. Malarkodi****, My Brother* ***Mr. S. Pranaav*** *for their care, love, support and prayers to overcome all my hardships and relieving me from responsibilities and giving way to make up with my course.*

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF GRAPHS

INTRODUCTION

INTRODUCTION

Diabetes is one of the major health issues in the 21st century and the number of people affected by diabetes worldwide in 2015 is estimated to be 415 million which may rise to 642 million by 2040. It is now epidemic in many developing countries and nearly 5 million have died from diabetes in 2015 with the prevalence of diabetes is estimated to be 1 in 11 adults. In India one in two adults is diagnosed with diabetes and the number of adults with diabetes is estimated to be 69.2 million following China [1, 2, 3].

Diabetes is a metabolic disorder that occurs when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces causing raised levels of glucose in the blood. There are 2 main types of diabetes based on its etiology, as Type 1and Type 2. Type 1 is an autoimmune disease which causes the destruction of beta cells of the pancreas leading to absolute insulin deficiency, whereas Type 2 is due to cellular dysfunction in resistance to insulin by peripheral tissue and is influenced by factors such as lifestyle, age, and obesity in addition to a strong genetic environment. Type 2 is the most common and it accounts for 90-95% all patients with diabetes [1, 3, 8, 10].

It has been shown that sustained hyperglycemia may cause damage to many tissues in the body leading to multisystem complications such as retinopathy, neuropathy, and macro/microangiopathy. Early diagnosis and regular monitoring are essential to prevent its devastating complications. The diagnosis and monitoring of DM are based on specific laboratory investigations and presence of clinical signs and symptoms. The current method of investigation is blood glucose test where blood is collected by needle prick or venipuncture which may cause unnecessary discomfort and mental trauma that may discourage the individuals to undergo the routine investigation. Thus there is need for noninvasive technique to diagnose and monitor the diabetes. For the past 10 years, studies have been done using

saliva as a non-invasive diagnostic tool for testing glucose and other parameters. Saliva offers some distinctive advantages as it can be collected non-invasively, and by individuals with limited training. No special equipment is needed for collection of the fluid [4, 5, 9, 11].

Saliva is the product of multiple salivary glands lying beneath the oral mucosa which can indicate local and systemic alterations. Each day, the human salivary glands produce almost 600 ml of serous and mucous saliva containing minerals, electrolytes, buffers, enzymes , enzyme inhibitors, growth factors, cytokines, immunoglobulins, mucin and other glycoproteins .These components of saliva can be related to the hormonal, immunologic, neurologic, nutritional and metabolic state of the individual .Glucose is a small organic molecule capable of moving easily through the membranes of blood vessels, passing from the blood plasma to the gingival fluid, through the gingival sulcus, reaching the saliva. In diabetic patients, the permeability of acinar cells of the parotid gland increases which provide an increase in the ultrafiltration of blood serum components such as glucose, amylase, and blood proteins. Therefore, an increase in blood glucose in the diabetic patients can cause higher levels of salivary glucose with the consequent loss of homeostasis and greater susceptibility to diseases in the oral cavity [5, 8, 13].

The most common oral complications of diabetes mellitus include periodontal diseases, caries, candidiasis, angular cheilitis, sialosis, and burning mouth syndrome. Most of the above mentioned complications are linked to xerostomia which is due to autonomic neuropathies, microvascular changes, hormonal imbalances, or a combination. Various studies have also shown that salivary glands are affected directly or indirectly in diabetes mellitus and it is also well known that diabetic patients are predisposed to having an increased density of candidal growth in the oral cavity, which can be related to poor glycemic control, neutrophil dysfunction, reduced salivary flow, and high glucose concentration in

blood and saliva and in medications [6, 9, 12]. Certain studies have shown that type 2 diabetic patients have increased levels of urea in saliva and salivary glucose was elevated only in patients with poorly controlled diabetes[13].

Hence the study was conducted to evaluate the degree of severity of xerostomia, the presence of salivary glucose, salivary urea, and oral candidal growth in an uncontrolled diabetic patient and compare the degree of severity of xerostomia, salivary glucose, salivary urea, and oral candidal count in the saliva between type 2 diabetic patients and non- diabetic patients.

AIMS AND OBJECTIVES

AIMS OF THE STUDY

To determine the degree of severity of xerostomia, the salivary concentration of glucose, urea and salivary candidal carriage in patients with type 2 diabetics and non-diabetics.

OBJECTIVE OF THE STUDY

The objective of the study is Comparison of xerostomia, salivary glucose, salivary urea and oral candidal count in the saliva of type 2 diabetic patients and non-diabetic patients.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Shehla Amer (2001)[15] conducted a study to compare the correlation of salivary glucose and blood glucose in type 2 diabetic patient and non-diabetic individuals. Serum and salivary glucose were assayed using GOD-PAP method. They concluded that there was the presence of glucose in the saliva of type 2 diabetic patients and none of the healthy individuals had glucose in the saliva. A significant correlation was found between salivary and blood concentrations in the diabetics.

BV Kumar (2005)[16] conducted a study to estimate the prevalence of Candida in the saliva of diabetic and non-diabetic subjects. The result showed that the Colonization and carriage of Candida in the oral cavity was found to be higher in diabetic subjects than in non-diabetic subjects. Colony Forming Unit of Candida in the oral cavity was greater in Type 1 diabetes mellitus when compared to Type 2 diabetes mellitus and non-diabetic subjects.

Carmen Carda (2006)[14] conducted a study to examine the biochemical properties of saliva and the presence of xerostomia in patients with type 2 diabetes mellitus. The results showed that there is increased the salivary level of urea and total salivary proteins. There was a decrease in microglobulin levels in saliva. Salivary glucose levels were increased only in patients with poorly controlled diabetes

Radhika sashikumar (2010)[16] performed a study to assess and evaluate salivary glucose levels in diabetic and non-diabetic subjects as well as the relationship between salivary glucose levels and salivary candidal carriage and they concluded that salivary glucose levels were significantly higher in diabetics than in non-diabetics and there was a significant positive correlation between salivary and plasma glucose levels. Candidal colony

forming units were significantly higher in diabetic subjects and showed a significant positive correlation with salivary glucose levels.

Panchbhai AS (2012)[5] conducted a study to assess the correlation of salivary glucose level with blood glucose level in people with diabetes mellitus. The salivary glucose was analyzed in unstimulated whole saliva samples using glucose oxidase method and concluded that the salivary glucose level there was a significant positive correlation to fasting blood glucose level in the uncontrolled diabetic group when compared to control group.

Panda Abikshyeet (2012)[11] conducted a study to compare the salivary glucose with blood glucose and glycosylated hemoglobin in healthy and newly diagnosed type 2 diabetes mellitus. Unstimulated saliva was collected from both groups as well. It was concluded as that there was highly significant correlation between serum glucose and salivary glucose in the diabetic patient and there was significant correlation between salivary glucose and HbA1c level in diabetic patients.

Ivanovski K (2012)[13] conducted a study to determine the degree of severity of xerostomia using a questionnaire. They also evaluated salivary glucose and urea in type 1 diabetic patient and compared it with the control group. They concluded that there is a significant correlation between the degree of xerostomia and the salivary level of glucose in diabetic patients.

Iraj Mirzaii-Dizgah (2013)[15] investigated the stimulated salivary glucose was collected from diabetic and non-diabetic subjects and glucose was determined by GOD-PAP assay and it was concluded that there was a significant positive correlation between serum

and saliva glucose concentration. They concluded that salivary glucose can be used as an alternative to serum glucose for diagnosis and monitoring of diabetes mellitus.

Prathibha KM (2013)[17] investigated the salivary parameters in diabetic and non-diabetic subjects. They compared the salivary flow rates and the salivary physical and biochemical parameters such as salivary pH, flow rate and organic and inorganic constituents in diabetic and non-diabetic subjects. The results were salivary pH, flow rate and salivary amylase were significantly lower in diabetics.whereas salivary glucose, and total proteins, sodium, and potassium were significantly higher in diabetics and lower levels of calcium in comparison to those in the non-diabetic group. They concluded that evaluation of salivary parameters can be used as a noninvasive alternative to serum parameters for screening, diagnosis and monitoring of diabetes mellitus.

Preethi Balan (2014)[18] assessed the unstimulated salivary and random non-fasting blood glucose level in type II diabetic and Control group using glucose oxidase method and concluded that salivary glucose levels were significantly higher in patients with diabetes than controls and there was a significant positive correlation between salivary and plasma glucose levels in patients with diabetes.

Kumar S (2014)[19] assessed and evaluated salivary glucose levels, blood glucose levels and oral candidal carriage using oral rinse technique in type II diabetic patients and control group and concluded that the salivary glucose levels were significantly higher in controlled and uncontrolled diabetics when compared with controls. The salivary candidal carriage was also significantly higher in uncontrolled diabetics when compared with controlled diabetics and non-diabetic controls.

Karki PC (2014)[20] correlated the fasting salivary glucose with fasting serum glucose and glycosylated hemoglobin in diabetes patients and healthy controls. Salivary and serum glucose was estimated by hexokinase method in autoanalyzer. Glycated hemoglobin (HbA1c) was analyzed by turbidimetric inhibition immunoassay in autoanalyzer. They concluded that the salivary glucose level was significantly higher in diabetic patients than the control group and there is a significant positive correlation between fasting salivary glucose and fasting serum glucose, whereas the correlation between fasting salivary glucose and glycated hemoglobin in diabetic patients was not significant.

Radhika (2014)[21] evaluated and compared the salivary flow rate and prevalence of xerostomia in diabetics and non-diabetics. Random non-fasting plasma glucose and glycosylated hemoglobin levels were used to determine the diabetic status of the individuals. Unstimulated saliva was collected using 'Spit technique'. Stimulated saliva was collected using 2% citric acid. Unstimulated and stimulated salivary flow rate was measured as ml/min. Xerostomia was evaluated using questionnaire. They concluded that stimulated and unstimulated salivary flow rates were decreased in diabetic group compared to control and a greater percentage of diabetic patient perceived xerostomia symptoms compared to nondiabetics.

Azizi (2014)[22] evaluated blood glucose levels and salivary glucose levels in type II diabetics and healthy control group and concluded that a high correlation was found between blood glucose level and salivary glucose in diabetic patients

Lamichhane RS (2015)[9] assessed the candidal carriage in type 2 diabetic and non-diabetic groups using oral rinse method. Sabouraud's dextrose with Chloramphenicol was

used as a growth medium. They concluded that there was a significant increase in candidal colonization and growth in oral cavity among the diabetic group when compared with the non-diabetic group.

Preethi Balan (2015) [23] assessed and evaluated salivary glucose, pH and candida carriage rate in the saliva of type 2 diabetic and non-diabetic (control) group and concluded that the diabetic group showed significantly increased candida carriage rate and it was positively correlated with the increased salivary glucose whereas negatively correlated with the salivary pH. Thus it was concluded that in diabetic group increase in salivary glucose favors the growth of candida.

Indira M (2015)[24] comparatively assessed the salivary glucose, amylase and total protein levels in Type 2 diabetes patients and controls and concluded that salivary glucose level was significantly higher whereas salivary amylase and total proteins were lower in diabetic group when compared to control group and they found that there is no significant correlation between salivary and blood glucose levels.

Vidya kadashetti (2015)[25] evaluated and correlated salivary glucose and random blood glucose between diabetic patient and non-diabetic patients and concluded that the salivary glucose levels was significantly higher in diabetics patients when compared to control group and also positively correlated with the plasma blood glucose levels.

Lavanya Kalapala (2015)[26] correlated salivary glucose, total protein and amylase levels, oral candidal carriage and strain diversity in type II diabetics and concluded that all salivary parameters and candidal carriage were significantly higher in uncontrolled type II

diabetes when compared to controlled diabetes and control group. Non-Candida albicans species such as C. tropicalis was isolated in higher frequency in uncontrolled diabetics and found that all the salivary parameters and candidal carriage were significantly correlated between diabetic states from healthy.

Lima-Aragao (2016)[27] evaluated salivary glucose, urea, calcium, total protein, amylase, secretory IgA, the IgA anti-Streptococcus mutans and anti-insulin IgA antibodies in diabetic patients and control group. Caries status was evaluated using the DMFT index and concluded that glucose, urea, calcium, anti-S.mutans IgA, total IgA, and anti-insulin IgA were significantly higher in diabetic patients but total protein and amylase levels were lower in these patients and there was no positive correlation between blood and salivary glucose levels in either group. Diabetic patients had a higher DMFT index.

Dhanya M (2016)[28] evaluated the relationship of fasting blood glucose level with fasting salivary glucose in type 2 diabetic and control group and concluded that there is an increase in the level of fasting salivary glucose in diabetics when compared to that of control group and found that there is significant positive correlation between fasting salivary glucose and serum glucose in both diabetic patients and in controls.

Dipti Soni Jaipuriar (2016)[29] measured and correlated salivary glucose and fasting blood glucose along with serum HbA1C in patients with type 2 DM and concluded that there is a significant correlation between fasting blood glucose and the salivary glucose and there is a positive correlation between HBA1c and Fasting Blood Sugar as well as salivary glucose.

Archana PS (2016)[7] evaluated serum glucose, serum calcium, serum potassium, serum sodium, along with salivary pH, salivary flow rate, and salivary glucose in type 2 diabetic and control group and concluded that there was decrease in salivary electrolyte and salivary calcium in uncontrolled diabetes when compared to controlled diabetes and control group. There was no significant difference between salivary pH and flow rate among the groups similarly no significant difference in serum sodium and potassium among the groups.

MATERIALS AND METHODS

MATERIALS AND METHODS

Study type: Observational study

Study design: Cross-sectional study

Study duration: October 2015 –October2016

Source of Data collection:

The size of the study sample consisted of 60 which were divided into 30 uncontrolled Type 2 diabetic and 30 Non-diabetic subjects. Subjects were of both sexes, who were 35-70 years suffering from diabetes and were attending the laboratory of a private hospital in Rasipuram, Tamil Nadu, India, between October 2015 to October 2016, after obtaining their informed consent.

INCLUSION CRITERIA

Patients aged between 35-70 yrs with clinically healthy oral mucosa.

Medical history of type II uncontrolled Diabetes mellitus for minimum of 2 years, and patients irrespective of whether they were under any medication for or not.

Diagnostic criteria for type II diabetes mellitus were as follows:

2 hour postprandial plasma glucose > 200mg/dl (11.1mM)

EXCLUSION CRITERIA

Patients who had the history of any other a systemic disease other than diabetes were excluded

Patients who were under medications for a systemic disease other than diabetes mellitus were excluded.

Patients who had the history of smoking and alcohol consumption were excluded.

Patient who had undergone surgery of the salivary glands were excluded.

Patient who had been exposed to radiation of the head and neck region were excluded.

Patient with poor oral hygiene were excluded.

Denture wearers were excluded

METHODOLOGY

The selection of the subjects would be based on their given past medical history and postprandial blood glucose level which would be measured during routine blood investigation. All the subjects were clinically examined to assess the oral hygiene and to exclude the possibility of any other oral disease or systemic disease with oral manifestation. Subjects of both the study and control groups were informed about the procedure and a written consent was obtained.

BLOOD COLLECTION AND ANALYSIS OF SAMPLE

Subjects were recruited when presenting for routine follow-up in the private hospital in the morning from 10 to 11 am, a postprandial venous blood sample (2ml) was collected, in an ethylene diamine tetra acetic acid containing blood collection tube. The test tube containing blood was centrifuged at 3500 rpm for 5 minutes, and then serum was used immediately for glucose detection. Serum glucose was analyzed by glucose oxidase method using the semiautomatic analyzer.

SALIVA COLLECTION AND ANALYSIS OF SAMPLE

Unstimulated mixed whole saliva was collected 2 hours after breakfast, from the study individuals sitting in upright position. Samples were collected by spitting method. The patients were asked to wash their mouths with tap water and were asked to spit two or three times, after which they were told to spit the saliva pooled in their mouths for the following 10 minutes into two separate sterile sample collection containers. For each subject 1ml of saliva was collected. Each sample was assayed for glucose and urea immediately or stored at -20°c in case of delay in analysis. Before analyzing, the sample was centrifuged at 3500 rpm for 5 minutes, and then the supernatant clear fluid was used for detection of glucose and urea.

GLUCOSE ANALYSIS

The quantitative estimation of postprandial salivary glucose was done by Glucose oxidase method using enzymatic kit GOD-POD, glucose oxidase-peroxidase.

PRINCIPLE OF THE ASSAY:

Glucose Oxidase oxidizes glucose to gluconic acid and hydrogen peroxide. In presence of enzyme peroxidase, released hydrogen peroxide is coupled with Phenol and 4-Aminoantipyrine (4-AAP) to form colored Quinoneimine dye. The absorbance of colored dye is measured at 505 nm and is directly proportional to glucose concentration in the sample.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + 4\ \text{Hydroxybenzoic acid} + 4\text{-amino antipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + 2H_2O$$

UREA ANALYSIS

The quantitative estimation of salivary urea was done by Modified Berthelot method

PRINCIPLE:

Urease catalyzes the conversion of urea to ammonia and carbon dioxide. The ammonia released reacts with a mixture of salicylate, Hypochlorite, and nitroprusside to yield a blue-green colored compound (Indophenol). The intensity of color produced is proportional to the concentration of urea in the sample and is measured photometrically at 578 nm.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO$$

$$NH_3 + \text{Salicylate} + \text{Hypochlorite} \xrightarrow{\text{Nitroprusside}} \text{2-2 – Dicarboxy Indophenol}$$

ASSESSMENT OF CANDIDAL COLONIZATION:

For assessing the candidal count the saliva sample containing the sterile container was the transported to the laboratory immediately or stored at -20°c in case of delay in analysis .An inoculation loop of 4mm of the saliva sample was taken and streaked onto culture plates containing Hicrome candida Differential Agar base, Modified M1297A/M1456A and incubated at 37°c for 48 hours.

PRINCIPLE AND INTERPRETATION

Perry and Miller reported that Candida albicans produce an enzyme ß-N-acetyl galactosaminidase and according to Rousselle et al incorporation of chromogenic or fluorogenic hexosaminidase substrates into the growth medium helps in identification of C.albicans isolates directly on primary isolation. Hicrome Candida Differential Agar is a selective and differential medium, which facilitates rapid isolation of yeasts from mixed cultures and allows differentiation of candida species namely C.albicans, C.krusei,

C.tropicalis, and C.glabarata on the basis of colouration and colony morphology. Peptic digest of animal tissue, peptone special, malt extract, and yeast extract provides nitrogenous, carbonaceous compounds and other essential growth nutrients. Phosphate buffers the medium well. Chloramphenicol suppresses the accompanying bacterial flora.

Organism	**Inoculum (CFU)**	**Growth**	**Colour of colony**
Candida albicans ATTC 10231	50-100	Good- luxuriant	Light green
Candida glabrata ATCC 15126	50-100	Good- luxuriant	Cream to white
Candida krusei ATCC 24408	50-100	Good- luxuriant	Purple, fuzzy
Candida tropicalis ATCC 750	50-100	Good- luxuriant	Blue to purple

Species were recognized based on color index given in The HiMedia Manual and the colonies were quantified based on calibrated loop method. They scoring were categorized as

1000- 20,000 –Insignificant

30,000- Significant

>1, 00,000 – Highly significant

QUESTIONNAIRE FOR ASSESSING SEVERITY OF XEROSTOMIA

To determine the degree of severity of xerostomia, the special questionnaire was given to the patients. The questionnaire was developed in regional language to get information of patient data like: age, sex, years of diabetes(less than 10 years, more than 10 years) and xerostomia existed or not as a subject in the sensation of dry mouth. In this study we used questionnaire recommended by Carda, 2006 [14]

ARMAMENTARIUM

SAMPLE COLLECTION

Disposable sterile container

Disposable gloves and mouth masks

Glucose oxidase kit

Urea Berthelot kit

Hicrome Candida Differential Agar Base modified M1297A/M14564 and inoculation loop.

FIGURE 1. ARMAMENTARIUM FOR COLLECTING SALIVA

FIGURE 2: GLUCOSE KIT

FIGURE 3: UREA BERTHELOT KIT

FIGURE 4: HICHROME CANDIDA DIFFERENTIAL AGAR MEDIUM WITH CALIBERATED INOCULATION LOOP

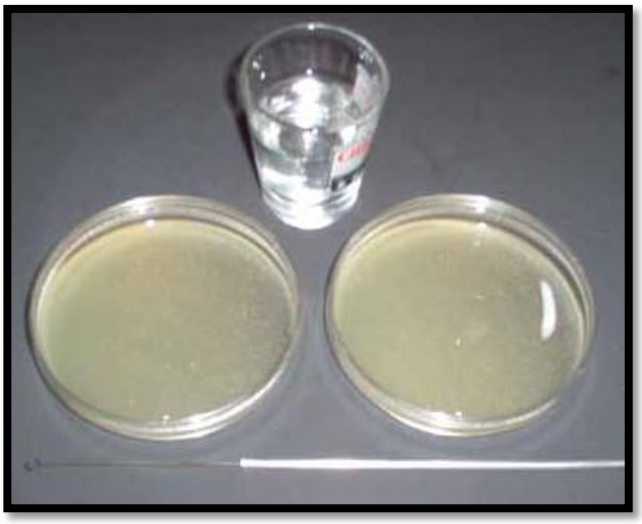


FIGURE 5: HICHROME CANDIDA DIFFERENTIAL AGAR SHOWS C.krusei and C.tropicalis species.

STATISTICAL ANALYSIS

STATISTICAL ANALYSIS

Data obtained was analyzed using Statistical package for Social Sciences (SPSS) software version 12.0. Intra and intergroup analysis was done using Pearson's Chi-square test and t-test. In the present study, the level of significance (α) was fixed at 5%. ($p \leq 0.05$). Comparison of salivary glucose levels and salivary urea levels between the control and patient groups were performed using *t*-test and chi-square test. Comparison of Oral candidal carriage rate in type 2 uncontrolled diabetic patients and control group was performed using a chi-squared test.

RESULTS

RESULTS:

In our study, 30 saliva samples were collected from uncontrolled type 2 diabetic and 30 saliva samples from non-diabetic group in the age group of 35- 70 yrs. Comparison of salivary glucose levels and salivary urea levels between the control and patient groups were performed using *t*-test and chi-square test. The mean serum glucose was significantly higher in an uncontrolled diabetic group than control group. Similar to the serum glucose levels, the salivary glucose levels, and the salivary urea levels in the uncontrolled diabetic group was significantly higher than the control group (Table 1).

We also compared the Oral candidal carriage growth in type 2 uncontrolled diabetic patients and control group using a chi-squared test. In uncontrolled diabetic patients 40% showed highly significant candidal growth, 50% showed significant candidal growth, 10% showed insignificant candidal growth. In control group 80% showed insignificant candidal growth and 20% showed significant candidal growth. The oral candidal carriage rate was highly significantly in uncontrolled diabetic patient group when compared to the control group (Table 2).

In the present study to know the severity of xerostomia among the uncontrolled diabetic and control group, we used a questionnaire and graded the severity as level 0 to 3 based on their response. In uncontrolled diabetic group, nearly 33.3% showed level 1 and another 33.3% did not have xerostomia whereas16.7% showed grade 2 and the remaining 16.7% showed grade 3.In Control group, 97.7% did not have xerostomia and 3.3% showed grade 1 .Thus severity of xerostomia in uncontrolled diabetic patient is significantly higher than the control group($p=0.000$).

Table 1: Distribution of blood glucose level, salivary glucose level, and salivary urea level between type 2 uncontrolled diabetic and non-diabetics control groups.

Groups	Number	Blood Sugar (mg/dl) Mean± SD	Salivary Sugar (mg/dl) Mean ±SD	Salivary Urea (mg/dl) Mean ±SD	P value
Uncontrolled Diabetic	30	299.3 ± 71.4	17.1 ± 3.4	59.3 ± 29.7	P=0.000
Non-Diabetic (Control)	30	105.6 ± 8.8	8.6 ± 0.9	27.5 ± 4.3	P=0.000

GRAPH 1: Distribution of blood glucose level, salivary glucose level, and salivary urea level between type 2 uncontrolled diabetic and non-diabetic control groups.

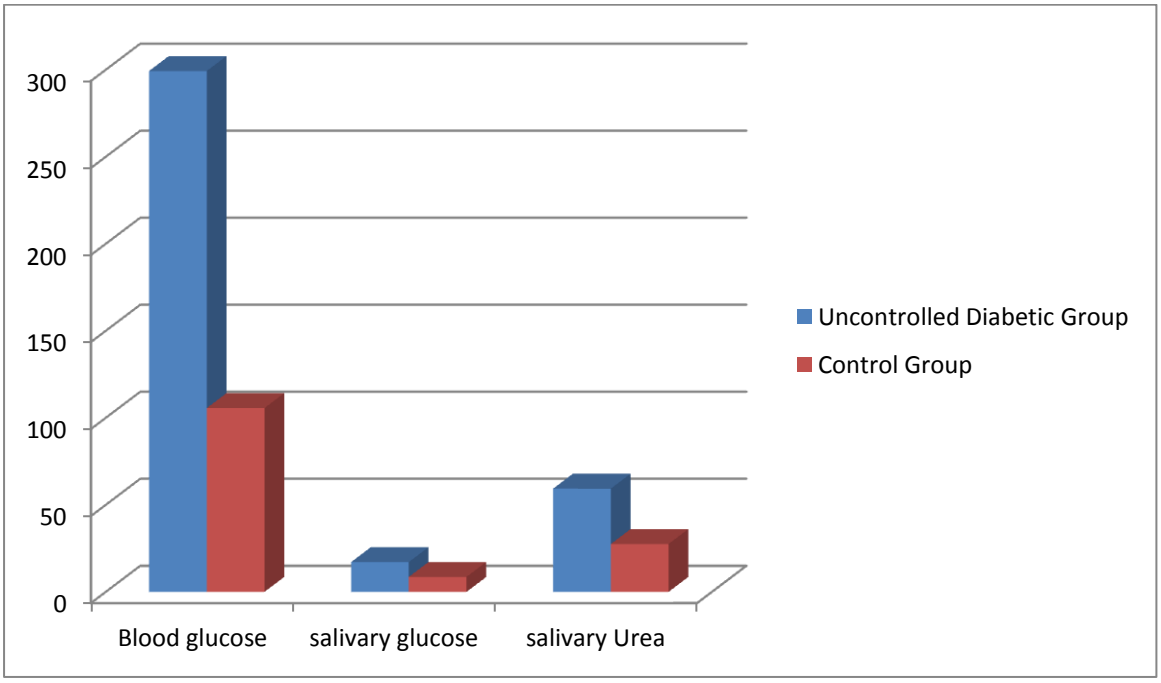


Table 2: Distribution of oral candida *c*arriage in type 2 uncontrolled diabetic and non-diabetic control groups

Groups	Highly significant		Significant		Insignificant	
	Number	Percent	Number	Percent	Number	Percent
Uncontrolled diabetic	12	40%	15	50%	3	10%
Non-diabetic (control)	0	0%	6	20%	24	80%

(P=0.000)

GRAPH 2: Distribution oral candida *c*arriage in type 2 diabetic and non-diabetic control groups

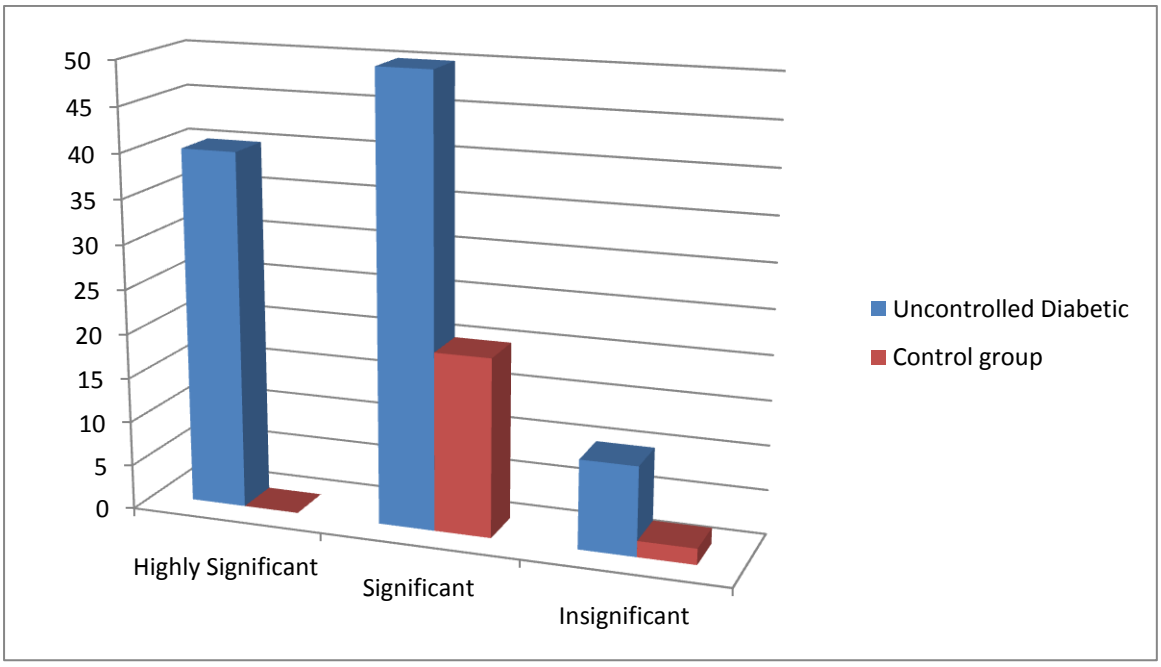
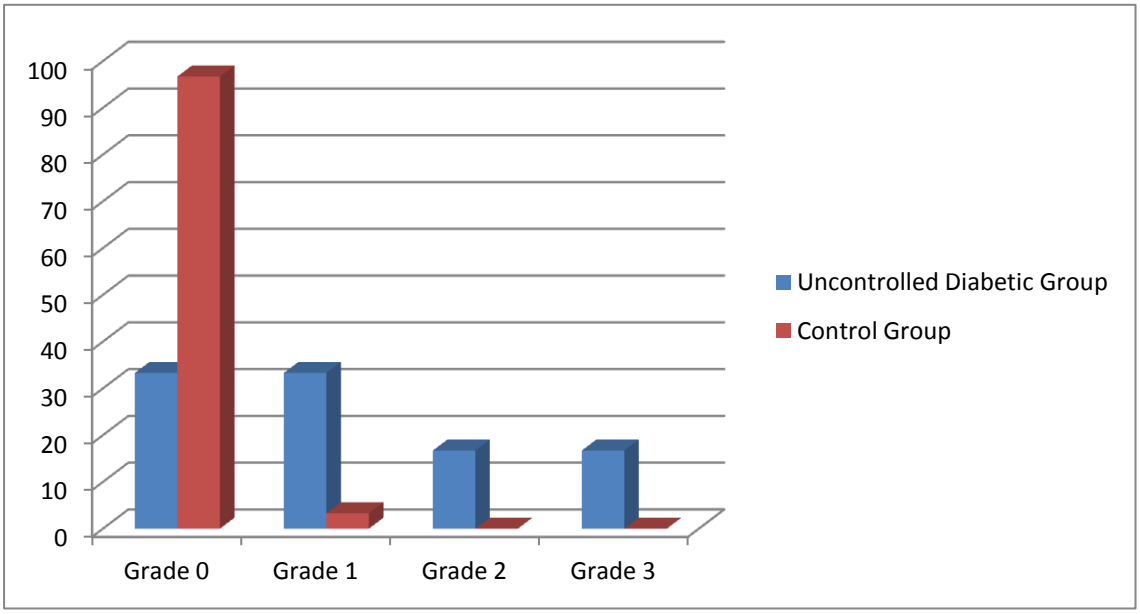


Table 3: Distribution of degree of severity of xerostomia in type 2 uncontrolled diabetic and non-diabetic control groups.

Xerostomia Level	Uncontrolled Diabetic		Control group		P value
	Number	%	Number	%	
Grade 0	10	33.3%	29	96.7%	
Grade 1	10	33.3%	1	3.3%	P=0.000
Grade 2	5	16.7%	0	0%	
Grade 3	5	16.7%	0	0%	

GRAPH 3: Distribution of degree of severity of xerostomia in type 2 uncontrolled diabetic and non-diabetic control groups.



DISCUSSION

DISCUSSION:

Saliva is a biological fluid that mirrors the body's state in health and disease. Many biochemical parameters of saliva have been noticed to have certain favorable factors over that of serum[31]. Murrah et al (1985) has proved that changes in basement membrane of the parotid gland could alter the ability of the glands to transfer molecules, electrolytes, and water resulting in an altered salivary output[21]. Glucose is an organic molecule that has the capability of moving easily through the membranes of blood vessels, and it can pass from the blood plasma to the gingival fluid, through the gingival sulcus, and can reach the saliva. In case of diabetic patients, there is an increase in the ultrafiltration of blood serum components such as glucose, amylase, electrolytes, and blood proteins and therefore, an increase in blood glucose in the diabetic patients can cause higher levels of salivary glucose with the consequent loss of homeostasis and greater susceptibility to diseases in the oral cavity [5, 8, 13].

The results in our study showed that there was an increase in the salivary glucose levels in uncontrolled type 2 diabetics when compared with non-diabetic healthy control group and there was an increase in the salivary urea and the oral candidal carriage in the uncontrolled diabetic group when compared to non-diabetic healthy control. Xerostomia was present in 66.7% in diabetic group and 3.3% in non-diabetic control group.

Various authors have mentioned that there was an increase in salivary glucose levels in type 2 diabetic patients when compared to non-diabetic healthy group which was similar to the result of our study[5,7,8,11,14,15,17,18,20,22,30]. Manjrekar (2012)[33] showed that salivary glucose did not differ significantly between the diabetic and nondiabetics groups which was in contrast to our study result.

Amer et al. (2001)[15] mentioned that the patient had an average blood glucose level over an extended period of time, and glucose was present only in the saliva of diabetic patients and non-diabetic patient did not show the presence of glucose in the saliva. Carda et al (2006)[14] showed that salivary glucose was only present in patients with poor glycemic control which was similar to our study and salivary urea and total proteins were also increased and microalbumina level was reduced in diabetic group.

Urea is a diamide of carbonic acid and it is an end product of protein catabolism in solutions behaves as a moderately alkaline compound and this compound has low molecular weight and it can easily pass through the membrane of the acinar cell through ultra filtration[13]. Salivary glands do not synthesize urea, but it reaches them through the ultra filtration of blood serum, on the level of acinar cells in the salivary glands.

Xerostomia is a subjective feeling of dryness in the mouth, which is caused by salivary hypofunction. Xerostomia symptoms are more frequent among the elderly population, but they are not directly associated with the increasing age of the individual and results from the negative effects of diabetes on the sympathetic and parasympathetic nervous systems, which play a key role in the secretion of saliva. Also, far greater dehydration of diabetics and hormonal changes are factors which cause decreased saliva production in these patients[13]. Similarly, Vasconcelos et al (2010)[8] showed that the salivary glucose concentration was significantly higher in the diabetic than non diabetic was and Xerostomia was reported in 12.5% of diabetic patients and 5% of non-diabetic patients.

Radhika (2014)[21] mentioned that the Type 2 diabetics have higher prevalence of xerostomia and reduced salivary flow rate which causes an imbalance in the homeostasis of oral environment leading to spectrum of oral ailments in these individuals.

Kumar S (2014)[19] and Lamichane et al (2015)[9] showed that there was an increase in the salivary glucose level and oral candidal carriage in the uncontrolled type 2 diabetics, which was similar to our study. A study performed by Hill et al (1989)[31] showed that very poorly controlled diabetics or uncontrolled diabetics are significantly more susceptible to oral candidiasis, which was similar to our study. Salivary glucose forms chemically reversible glycosylation products with proteins in tissues during hyperglycemic episodes, and this leads to the accumulation of glycosylation products on the buccal epithelial cells, which in turn may increase the number of available receptors for Candida. This finding is suggestive of the fact that uncontrolled or poorly controlled diabetes increases the susceptibility to oral opportunistic infections, such as oral candidiasis.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION:

The present study consisted of 60 saliva samples which were divided into 30 uncontrolled Type 2 diabetic and 30 Non-diabetic subjects. Subjects were of both sexes, who were 35-70 years suffering from diabetes and were attending the laboratory of a private hospital in Rasipuram, Tamil Nadu, India, after obtaining their informed consent. The saliva was collected using spitting method. The method used to determine the blood and salivary glucose was glucose oxidase peroxidase and for estimation of salivary urea was done by Modified Berthelot method**.** Hicrome Candida Differential Agar Base, modified M1297A/M14564 was used to assess the candidal colonization using an inoculation loop method. Xerostomia severity was evaluated using questionnaire recommended by Carda et al[14].In the uncontrolled diabetic group (N=30) the mean serum glucose was estimated to be 299.3 ± 71.4mg/dl and the mean for the control group (N=30) was 105.6 ± 8.8mg/dl. The oral candidal carriage rate in the uncontrolled diabetic group was assessed as 40% has showed highly significant candidal growth, 50% has showed significant candidal growth, and 10% has showed insignificant candidal growth. In the control group 80% showed insignificant candidal growth and 20% showed significant candidal growth. The degree of xerostomia in the uncontrolled diabetic group showed nearly 33.3% has grade 1 xerostomia, 16.7% showed grade 2, 16.7% showed grade 3 and 33.3% did not have xerostomia. In Control group, 97.7% did not have xerostomia and 3.3% showed grade1.66.7 % of uncontrolled diabetic patients has xerostomia.

Based on the results of this study we conclude that the degree of xerostomia, the salivary glucose level, the salivary urea level, and the oral candidal carriage were significantly higher in uncontrolled type 2 diabetic group when compared to the non-diabetic healthy control group. We conclude that salivary glucose level can be used as an alternative method for diagnosis and monitoring the diabetes mellitus.

LIMITATION OF THE STUDY:

Study samples were small and large samples are needed for intra group analysis.

The method used for estimation of glucose was glucose peroxidase. Other methods can also be analyzed like hexokinase method which may influence the results.

BIBLIOGRAPHY

BIBLIOGRAPHY:

1. David cavan, Fernandes JR, Markarozz L, Ogustsova K.Diabetes Atlas 7th ed International Diabetes Federation 2015: 13-16.
2. Global Report on Diabetes 2016.Geneva:World health Organisation; 2016.
3. Micheal Glick. Burket's oral medicine. 12th ed. PMPH-USA, 2015: 567-568.
4. Eliaz Kaufma, Ira B. Lamster. The diagnostic applications of saliva-A review. Crit rev Oral Biol Med 2002; 13(12):197-212.
5. Panchbhai AS. Correlation of salivary glucose level with Blood glucose Level in Diabetes Mellitus. J Oral Maxillofac Res 2012; 3(3):e3.
6. Vijaylaxmi B Madalli, shrinivas M Basavarradi, Krishna Burde, Priya Horatti . Saliva- A Diagnostic tool OSR-JDMS. 2013; 6: 96-99.
7. Archana PS, Gopal KS, Vardhan BGH, Kumar PM. Saliva as a Non-invasive Tool in Evaluation of Type 2 Diabetes Mellitus. Int J Sci Stud 2016;4(1):178-182.
8. Vasconcelos AC, Soares MS, Almeida PC, Soares TC. Comparative study of the concentration of salivary and blood glucose in type 2 diabetic patients. J Oral Sci 2010; 52(2):293-298.
9. Lamichane Rs, Boaz K, Natarajan S, Shrestha M.A. Assessment of candidal carriage in patients with type II Diabetes Mellitus. Journal of Pathology of Nepal 2015;5, 733-738.
10. Aitken- Saavedra J et al. Salivary gland dysfunction markers in type 2 diabetes mellitus patient. J Clin Exp Dent. 2015; 7(4):e501-505.
11. Abikshyeet et al, Glucose estimation in the salivary secretion of diabetes mellitus patients. Diabetes, Metabolic Syndrome and obesity: targets and therapy 2012:5 149-154.
12. Premkumar J, Ramani P, Xhandrasekar T, Natesan A, Premkumar P. Detection of species diversity in oral candida colonization and antifungal susceptibility among non oral habit adult diabetic patients. J Nat Sc Biol Med 2014; 5: 148-154.

13. Ivanovski K, et al. Xerostomia and salivary levels of glucose and urea in patients with diabetes. Contributions, Sec. Biol. Med. Sci.2012: 219–229.
14. Carda C, Mosquera-Lloreda N, Salom L, Gomez de Ferraris ME, Peydro A. Structural and functional salivary disorders in type 2 diabetic patients. Med Oral Patol Oral Cir Bucal 2006; 11:e309-314.
15. Mirzai- Dizgah I et al. Salivary glucose can be used as an alternative to serum glucose for diagnosis and monitoring of diabetes mellitus. J Arch Mil Med, 2013; 1(1): 24-27.
16. Sashikumar R, Kannan R, Salivary glucose levels and oral candidal carriage in type II diabetics. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109: 706-711.
17. Prathibha.K.M et al., Evaluation of Salivary Profile among Adult Type 2 Diabetes Mellitus Patients in South India. Journal of Clinical and Diagnostic Research. 2013; 7(8): 1592-1595.
18. Balan P, Babu SG, Sucheta KN, Shetty SR, Rangare AL, Castelino RL,et al. Can saliva offer an advantage in monitoring of diabetes mellitus? –A case control study. J Clin Exp Dent, 2014; 6(4):e335-338.
19. Kumar S, Padmashree S, Jayalekshmi R.Correlation of salivary glucose, blood glucose and oral candidal carriage in the saliva of type 2 diabetics: A case-control study. Contemp Clin Dent 2014; 5: 312-317.

20. Karki PC, Khan SA, Chaudhari RK, Rajendra KC. Salivary glucose level in diabetic patients: A comparative cross sectional study. Int Journal of Therapeutic Applications, 2014;18: 12-17

21. Radhika T, Ranganathan k. Salivary Output in Type 2 Diabetic Patients .Oral Maxillofac Pathol J, 2014; 5(1):413-417.

22. Azizi et. al. The Correlation of Blood Glucose with Salivary glucose Level in Diabetic Patients. Journal of Islamic Dental Association of Iran 2014; 26, (1).

23. Balan P et al. Candida Carriage Rate and Growth Characteristics of Saliva in Diabetes Mellitus Patients: A Case–Control Study. J Dent Res Dent Clin Dent Prospect 2015; 9(4):274-279.

24. Indira M, Chandrashekar P, Kattappagari KK, Chandra LK, Chitturi RT, Ramana Reddy BV. Evaluation of salivary glucose, amylase, and total protein in Type 2 diabetes mellitus patients. Indian J Dent Res 2015; 26: 271-275.

25. Vidya kadashetti et al. Glucose Level Estimation in Diabetes Mellitus By Saliva: A Bloodless Revolution. Rom. J. Intern. Med., 2015, 53, (*3*), 248–252.

26. Lavanya Kalapala, Vandana Raghunath, Ajay Bernard Reginald. Salivary Factors, Candidal colonization and Strain Diversity in Type II Diabetics. National Journal of Laboratory medicine. 2015, 4(4): 52-58

27. Lima‑Aragao *et al.* Salivary profile in diabetic patients: biochemical and immunological evaluation. BMC Res Notes 2016;9:103

28. Dhanya M, Hegde S. Salivary glucose as a diagnostic tool in Type II diabetes mellitus: A case-control study. Niger J Clin Pract 2016;19: 486-490.

29. Dipti Soni Jaipuriar, Arun Kumar Singh , Shivadhar Sharma. Salivary and Serum Glucose in relation to HbA1c levels in Type 2 Diabetes Mellitus. SMU Medical Journal,2016;3 (1):638-649

30. Patel BJ et al. Comparison and correlation of Glucose levels in serum and saliva of both Diabetic and Non- diabetic patients. Journal of International Oral Health 2015; 7(8):70-76.

31. Maitreyee DS et al. A correlative study of salivary and plasma glucose levels in type 2 diabetic patients. International Journal of Clinical Biochemistry and Research 2016;3(2):250-254

32. Hill LV, Tan MH, Pereira LH, Embil JA. Association of oral candidiasis with diabetic control.J ClinPathol1989; 42: 502‑505.

33. Mahdavi SO, Hashemi S, Boostani N, Zokaee H .A New Method to Evaluate Fasting Plasma Glucose by Salivary Glucose Measurement. Iran J Diabetes Obes 2012; 4(3): 127–133.

ANNEXURE

ANNEXURE-I

QUESTIONNAIRE FOR ASSESSING SEVERITY OF XEROSTOMIA

Name: Age:

Sex: 1. Male 2.Female Address:

Question A: Have you had a feeling of dryness in the mouth in the last 6 months?

Yes/no.

Question B: How much saliva do you have in your mouth?

Little / enough / a lot.

Question C: Do you have difficulty swallowing food?

Yes/no.

Question D: Do you have to take liquid to facilitate swallowing food? Yes/no.

Based on the answers to these questions we determined the degree of severity of xerostomia.

Xerostomia 1: only when there is a positive answer to question A.

Xerostomia 2: when there is a positive answer to question A and one positive response (B, C or D).

Xerostomia 3: when there is a positive answer to question A and two other positive responses (B, C, or D)

ANNEXURE-II

வாய் உலர்ந்த தன்மையை பரிசீலிக்கும் கேள்விகள்.

பெயர்:

வயது:

பாலினம்: 1.ஆண் 2. பெண்

ஊர்:

நீரிழிவு நோய் உள்ளதா: 1.ஆம் 2. இல்லை

ஏவ்வளவு காலமாக நீரிழிவு நோய் உள்ளது:

1. 10 வருடத்திற்குள் நீரிழிவு நோய் உள்ளது

2. 10 வருடத்திற்கு மேல் நீரிழிவு நோய் உள்ளது.

சிகிச்சை எடுத்துக்கொண்டு இருக்கும் முறை: 1. மாத்திரை / 2. ஊசி

மருத்துவர் அறிவுரைப்படி மருந்தை தொடர்ந்து எடுத்து கொள்கிறீர்களா:

1. ஆம் 2. இல்லை

வாய் உலர்ந்த தன்மையை பரிசீலிக்கும் கேள்விகள்

1. கடந்த 6 மாதங்களாக வாய் உலர்ந்த தன்மை உள்ளதா?
   (ஆம் / இல்லை)

2. வாயில் உமிழ்நீர் எவ்வளவு அளவு உள்ளதாக கருதுகிறீர்கள்?
   (சிறிது அளவு /போதுமான அளவு /அதிக அளவு)

3. உணவு விழுங்குவதில் ஏதும் சிரமம் உள்ளதா?
   (ஆம்/இல்லை)

4. உணவு விழுங்க தண்ணீர் தேவைப்படுகிறதா?
   (ஆம்/இல்லை)

ANNEXURE-III

INFORMED CONSENT FORM

I ……. hereby declare that I clearly understood the procedures of the study. Also, I declare that I give permission for the above mentioned individual/organization/hospital to do the procedure to the individual/organization listed above.

Signature ……. Date……..

I have explained the above and answered all questions asked by the participant.

Signature…….. Date……

ANNEXURE-IV

ஒப்புகை வாக்குமூலம்

..... ஆகிய நான் மேற்கூறிய ஆராய்ச்சி படிப்பின் வழிமுறைகளைத் தெளிவாகப் புரிந்து கொண்டேன். மேலும் நான் இந்த ஆராய்ச்சிப் படிப்புக்கான வழிமுறைகளை மேற்கொள்வதற்கும், அதன் பரிசோதனை முடிவுகளை தெரிந்து கொள்ளவும் முழுமையாக அனுமதிக்கிறேன்.

.....

நோயாளியின் கையொப்பம் தேதி.....

நான் மேற்கூறிய ஆராய்ச்சிப் படிப்பிற்கான விதிமுறைகள் மற்றும் அது குறித்த நோயாளியின் சந்தேகங்களையும் தெளிவாக விளக்கியுள்ளேன்.

.....

மருத்துவரின் கையொப்பம் தேதி.....

ANNEXURE V

INSTITUTIONAL ETHICAL COMMITTEE

KSR INSTITUTE OF DENTAL SCIENCE & RESEARCH
KSR Kalvi Nagar, Tiruchengode-637 215, Tamilnadu.
Phone : 04288-274981, Fax : 04288-274761,
email : ksrdentalcollege@yahoo.com

Ref.: 089 /KSRIDSR/EC/2014 **Date : 22.12.2014**

To

Dr.S.Priyanka,
Postgraduate Student,
Dept. of Oral Medicine & Radiology,
KSR Institute of Dental Science & Research,

*****

Your dissertational study titled "COMPARISON OF XEROSTOMIA, SALIVARY LEVELS OF GLUCOSE, UREA AND ORAL CANDIDAL CARRIAGE IN THE SALIVA OF TYPE 2 DIABETICS AND NON DIABETICS: A CROSS SECTIONAL STUDY" presented before the ethical committee on 17th Dec.2014 has been discussed by the committee members and has been approved.

You are requested to adhere to the ICMR guidelines on Biomedical Research and follow good clinical practice. You are requested to inform the progress of work from time to time and submit a final report on the completion of study.

Signature of Member Secretary
(Dr.G.S.Kumar)

PRINCIPAL,
K.S.R. INSTITUTE OF DENTAL
SCIENCE & RESEARCH,
K.S.R. KALVI NAGAR,
THOKKAVADI POST,
TIRUCHENGODE - 637 215